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THE ASSIMILATION OF PROTEIN AND CARBOHYDRATE FROM PREPARED DIETS BY THE SHRIMP, *Penaeus stylirostris*

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ABSTRACT

Assimilation (apparent digestibility and absorption) of protein and carbohydrate from selected diets was tested using *Penaeus stylirostris*. The largest shrimp (approximately 10 g) assimilated squid meal proteins better than α-soy protein but smaller shrimp (1.3 and 3.8 g) did not. All sizes assimilated protein from diets containing α-soy better than those containing brewer's yeast. Carbohydrate assimilation was less efficient than protein for all diets. There was no relation between carbohydrate and protein assimilation and the percentage in the diets over the limited range tested. Although assimilation of these two groups of compounds was greater in the larger shrimp, there was no correlation between the growth and protein and carbohydrate assimilation for the diets tested for similar size animals of the same species.

INTRODUCTION

Growth is only one of several possible parameters that can be used to optimize shrimp diets. As compared to growth experiments, assimilation efficiency experiments are shorter and less expensive. Assimilation data along with growth, survival and food conversion data give additional understanding of the nutritional efficiency of prepared rations.

Assimilation, which is a measurement of apparent digestibility and absorption, of the components of a formulated feed are difficult to determine directly for aquatic animals because of problems in measuring the total amount of ingestion and defecation. However, relative assimilation of feeds and their components can be indirectly determined by adding chromic oxide (Cr_2O_3) as a reference component in rations (McGinnis and Kasting 1964a,b; Nose 1964). Chromic oxide is physiologically inert, non-toxic and can be easily mixed in formulated feeds. This technique has been used with success to measure apparent digestibility and absorption of several components of diets such as proteins, carbohydrates, lipids, minerals, amino acids, sterols, etc., in shrimps and prawns (Nose 1964; Ting 1970; Lee 1970, 1971; Forster and Gabbott 1971; Forster 1972; Teshima et al. 1974; Deshimaru 1976; Colvin 1976).

In the present paper, the chromic oxide method was employed to determine relative assimilation of proteins and carbohydrates from selected diets for different sizes of pond-reared *Penaeus stylirostris*.

MATERIALS AND METHODS

Penaeus stylirostris were obtained from ponds at the Texas A&M University Shrimp Mariculture Facility at Corpus Christi. Four sizes, 1.2-1.4 g, 3.4-4.0 g, 9.1-9.7 g, and 9.8-11.9 g (ranges of mean weights tested), were stocked at a density of 25, 20, 15 and 22 shrimp per aquarium, respectively. Water temperatures and salinities for these experiments ranged between 26-29°C and 19-24 ppt, respectively.

Assimilation efficiency experiments were conducted in specially modified glass aquaria (0.72 m^2 bottom) with volumes of 520 liters. The aquaria were divided into two areas. One, the "feeding" area, was approximately 1/4 of the bottom area and was prepared with an undergravel filter bed of crushed oyster shell. The other, the "collection" area, did not have substrate and was separated from the "feeding" area by a movable plastic mesh barrier.

Shrimp received from Corpus Christi were put into the glass aquaria, fed Ralston Purina MR 25 ration for 4 days and then fasted the day prior to the initiation of the experiment. For the tests, the shrimp were moved each morning to the gravel area where they were fed different diets (5% of biomass) for one hour while the "collection" area was cleaned of feces and exuviae. Although the first colored feces appeared between 15 and 50 minutes after introducing the feed, the shrimp were held in the "feeding" area for one hour before being moved gently to the "collection" area. After 6 hours in the "collection" area the shrimp were again transferred to the "feeding" area while feces were collected with a net. Feces were gently rinsed with distilled water to remove inorganic material and frozen at -20°C. This procedure was repeated for 5 consecutive days in each assimilation trial with the feces for each day analyzed separately.

Collected feces were dried at 60°C in an oven to constant weight for chemical determinations. Feces, prepared feed and meals were analyzed for protein and carbohydrate. The protein was estimated by determining the total nitrogen by the Kjeldahl method (semi-micro) according to Barnes (1959) and multiplying the total nitrogen value by 6.25. Carbohydrate was measured by the phenol-sulphuric acid method (Dubois et al. 1956) following digestion with 5% trichloroacetic acid (TCA). Total lipids in the diets were analyzed using the technique described by Hanson and Olley (1963), an adaptation of the Bligh and Dyer (1959) method.

Chromic oxide level in diets and feces was determined by first oxidizing Cr_2O_3 to Cr_2O_7 with perchloric acid, followed by colorimetric

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analysis of the dichromate ion with diphenylcarbazide (McGinnis and Kasting 1974a,b). All chemical determinations were run in duplicate or triplicate and are reported on dry weight basis.

The relative assimilation of proteins or carbohydrates was calculated according to the equation:

$$\text{relative assimilation efficiency (\%)} = (100) - (100)\left[\frac{(\%\ Cr_2O_3 \text{ in feed})}{(\%\ Cr_2O_3 \text{ in feces})} \times \frac{(\%\ \text{nutrient in feces})}{(\%\ \text{nutrient in feed})}\right]$$

For the evaluation of significant differences in assimilation between diets the following statistical tests were performed: Cochran or Bartlett tests for homoscedasticity of variances (Gmurman 1974); analysis of variance (F_s reported in tables for homoscedastic groups) and Student's t test between diets. Analyses of covariance were performed to assess the relation of the sizes of shrimp to protein and carbohydrate assimilation.

DESIGN OF THE FEEDS

Diets tested in these assimilation trials had the same composition as those used in the growth experiments reported by Fenucci et al. (1980, 1981), differing only in the replacement of 1% of rice bran by the same percent of Cr_2O_3. The organic composition of the main ingredients of the rations is shown in Table 1.

Table 1. Percent Organic Composition of the Principal Ingredients of the Prepared Feeds

Constituent	Protein	Carbohydrate	Lipid
Squid meal	76.5	0.9	15.8
α-soy	80.7	1.0	0.3
Brewer's yeast	40.0	44.3	0.9
Shrimp meal	41.0	1.0	5.4
Fish meal	62.7	1.2	12.2
Rice bran	16.4	28.2	6.0
Fish solubles	60.0	14.0	12.0

Pelletized diets were divided in two groups. In the first group, diets 1 to 4, different percentages of squid meal and α-soy were used, keeping the other components of the diets constant (Table 2). Diet 1 contained 12.7% squid meal, diet 3 had 12.4% α-soy, diet 2 had 6.2% α-soy and 6.4% squid meal and diet 4 had 3.2% squid meal and 9.3% of α-soy meal. In diet 18, all the α-soy and 15.2% of the rice bran of diet 2 were replaced by brewer's yeast. The second series of diets was based on diet K. In diets 25 and 26, 15.9 and 52.2% respectively of the shrimp meal contained by diet K were replaced by the same percent of brewer's yeast. A commercially available diet (11-B) of unknown composition ground and rebound with 1.0% of sodium alginate, 1.0% sodium hexametaphosphate and 1% of Cr_2O_3 was also tested. This commercial diet is an

adequate feed for raising shrimp in ponds (Chamberlain et al. 1981) and served as control diet with which the experimental diets were compared.

Table 2. Percent Composition of Formulated Rations Tested for Assimilation of Protein and Carbohydrate by *P. stylirostris*. The standard commercial diet (11-B) contained 22.1% protein, 4.5% lipid, 45.1% carbohydrate and 1.3% Cr_2O_3.

Constituent[b]	Diet[a]							
	1	2	3	4	18	K	25	26
Squid meal	12.7	6.4	--	3.2	6.4	5.0	5.0	5.0
α-soy	--	6.2	12.4	9.3	--	3.0	3.0	3.0
Brewer's yeast	--	--	--	--	12.5	--	5.0	15.8
Shrimp meal	28.4	28.4	28.4	28.4	28.4	31.5	26.5	15.7
Rice bran	41.4	41.5	41.7	41.6	35.2	43.0	43.0	43.0
Cr_2O_3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
% Protein	36.3	38.3	38.7	32.3	35.9	35.0	33.7	32.6
% Lipid	7.6	6.2	5.4	5.9	5.3	4.5	6.1	6.2
% Carbohydrate	17.9	14.9	17.0	15.9	20.8	15.9	22.0	22.9
% Cr_2O_3	1.2	1.2	1.2	1.4	1.8	1.8	1.5	1.8

[a]All diets contained fish meal, 8.0%; fish solubles, 2.0%; vitamin mix, 2.0%; alginate, 2.5%; Sodium hexametaphosphate, 1.0%; lecithin, 1.0%.

[b]Source of ingredients as in Fenucci et al. (1980).

RESULTS

Two preliminary experiments were conducted to evaluate possible sources of error for determining assimilation efficiencies. One was performed to determine the change in weight of feed during the maximum time that the prepared diets remained in water. Three 10 g samples kept in distilled water averaged a 2.28% (SE = ±0.08) loss while samples kept in sea water gained an average of 2.78% (SE = ±0.57). This gain in weight was probably due to absorption of sea water salts by the sample. Thus, these data indicated that there were no significant sources of error for the determination of assimilation efficiencies due to substances leaching from prepared feeds.

The other preliminary experiment was performed to determine if significant amounts of chromic oxide, protein and/or carbohydrates leached from the fecal pellets while they were immersed in sea water. Feces from 10 g animals (*P. stylirostris*) were analyzed 15 minutes, 1 hour and 6 hours after defecation and protein, carbohydrate and chromic oxide content of the feces showed no significant decrease with the time of immersion in sea water (Table 3). This indicated that there were no significant errors in the determination of the assimilation efficiencies due to chromic oxide, protein and carbohydrate loss from the feces.

Two assimilation experiments were conducted with large *P. stylirostris*. In the first experiment, diets 1, 2, 3, 4 and K were tested with animals averaging from 9.1 to 9.7 g mean weight (Table 4). Protein assimilation of these feeds varied between 89.4 and 82.7%. Assimilation efficiencies for diets K (89.4%) and 1 (88.5%) were significantly higher than values obtained for diets 4 (82.7%) and 3 (83.7%). Carbohydrate assimilation ranged from 71.7% in animals fed diet K to 57.0% for those fed diet 2 (Table 4). Carbohydrate assimilations for diets K (71.7%) and 1 (70.8%) were significantly higher than that of diets 3 (60.9%) or 2 (57.0%).

Table 3. Percent of Cr_2O_3, Protein and Carbohydrates in Feces of 10 g *P. stylirostris*, after Different Amount of Time in Sea Water

Constituent	Time in sea water		
	15 minutes	1 hour	6 hours
% Cr_2O_3	2.4 ± 0.22	2.4 ± 0.17	2.3 ± 0.22
% Protein	11.5 ± 0.44	11.0 ± 0.47	11.0 ± 0.47
% Carbohydrate	9.5 ± 0.51	9.3 ± 0.62	8.7 ± 0.37

Table 4. Relative Assimilation Efficiency of Crude Protein and Carbohydrate from Diets 1, 2, 3, 4 and K Fed to *P. stylirostris*. Values are means ± SEM for number of observations indicated.

Diet	Mean weight (g)	Number of observations[a]	Protein assimilation (%)	Number of observations[a]	Carbohydrate assimilation (%)
1	9.1 ± 0.36	4	88.5 ± 0.21	5	70.8 ± 2.80
2	9.2 ± 0.33	5	87.0 ± 1.20	4	57.0 ± 1.07
3	9.1 ± 0.44	4	83.7 ± 1.70	4	60.9 ± 2.97
4	9.5 ± 0.47	3	82.7 ± 1.84	3	60.7 ± 3.71
K	9.7 ± 0.39	4	89.4 ± 0.64	5	71.7 ± 0.60
			F_s = 5.28*		F_s = 6.32*

[a]There were 15 shrimp tested per observation.

* = significant ($P < 0.05$).

In the second experiment, the previous diets as well as diet 18 containing 12.5% brewer's yeast were tested with shrimp of 9.8-11.9 g mean weight (Table 5). Diet 1, containing 12.7% squid meal and no α-soy, gave significantly better protein assimilation (93.3%) than any of the other feeds. Protein assimilation for diet 18 (70.9%), containing 12.5% brewer's yeast, was significantly lower than all other feeds tested. Carbohydrate assimilation was greatest for diet 1 (82.3%) and least for diet 2 (45.5%). Both values were significantly different from those for diets K, 3, 4 and 18 (Table 5).

Two assimilation experiments also were conducted with smaller animals. The first experiment tested diets 1, 2, 3, 4 and K with shrimp having mean weights 3.4 to 4.0 g (Table 6). Protein assimilation ranged from 78.1 to 85.2%. Assimilation for shrimps fed diet 4 (78.1%) was

statistically lower than all other diets. Carbohydrate assimilation ranged from 57.5 to 66.4%. Although variances are heteroscedastic some pairwise comparisons for carbohydrate assimilation can be made. Assimilation of diet 1 was higher than diets 2 and 3 while lower apparent digestion and absorption found with diets K and 4 were not significantly different, probably due to the large variances observed for the replicates fed these diets.

Table 5. Relative Assimilation of Crude Protein and Carbohydrate from Diets 1, 2, 3, 4, K and 18 Fed to *P. stylirostris*. Values are means ± SEM for number of observations indicated.

Diet	Mean weight (g)	Number of observations[a]	Protein assimilation (%)	Number of observations[a]	Carbohydrate assimilation (%)
1	11.9 ± 0.49	5	93.3 ± 1.28	5	82.3 ± 2.53
2	10.7 ± 0.71	5	83.3 ± 2.15	3	45.5 ± 0.90
3	10.7 ± 0.73	5	85.8 ± 1.01	4	71.5 ± 3.10
4	9.8 ± 0.73	4	81.3 ± 3.24	4	63.4 ± 2.46
K	10.2 ± 0.63	5	81.5 ± 2.37	5	62.5 ± 2.68
18	10.6 ± 0.82	5	70.9 ± 2.41	4	66.3 ± 2.24
			F_s = 14.11*		F_s = 20.08*

[a]There were 22 shrimp tested per observation.

* = significant ($P < 0.05$).

Table 6. Relative Assimilation of Crude Protein and Carbohydrate from Diets 1, 2, 3, 4 and K fed to *P. stylirostris*. Values are means ± SEM for number of observations[a] in parentheses.

Diet	Mean weight (g)		Protein assimilation (%)			Carbohydrate assimilation (%)			After 7 days adjustment to diets					
									Protein assimilation (%)			Carbohydrate assimilation (%)		
1	3.8	0.16	83.1	1.20	(5)	66.4	1.15	(5)	87.2	1.33	(4)	70.7	1.05	(3)
2	4.0	0.24	85.2	2.13	(5)	57.7	1.36	(3)	87.9	1.49	(4)	68.6	2.29	(4)
3	3.4	0.26	84.8	1.31	(5)	59.4	0.89	(4)	86.2	0.54	(4)	71.9	2.36	(4)
4	3.8	0.17	78.1	1.56	(5)	57.5	5.86	(4)	86.1	1.10	(2)	52.7	3.11	(3)
K	3.8	0.27	82.2	1.30	(5)	58.9	5.85	(2)	88.4	2.28	(4)	66.2	3.60	(3)
			F_s = 3.47*			Heteroscedastic			F_s = 0.39[ns]			F_s = 8.37*		

[a]Twenty shrimp were tested per observation.

* = significant ($P < 0.05$); ns = not significant.

These shrimp were fed the same diet for an additional seven days after the first 5-day experimental period (Table 6) to check for potential changes in assimilation efficiencies with time. Protein assimilation for all diets showed a small increase compared to the first 5-day experimental period. This was significant only for diet 4. Following this period of adaptation, there were no significant differences in protein assimilation among diets. After seven days of adjustment, significant increases in carbohydrate assimilation were found for diet 1 (from 66.4% to 70.7%), 2 (from 57.7% to 68.6%) and 3 (from 59.4% to 71.9%). Assimilation of carbohydrates was significantly lower from diet 4 (52.7%) than from all the other diets (66.2% to 71.9%) after seven days of adjustment.

In the second experiment (Table 7), diets 1, 3, K, 18, 11-B, 25 and 26 were tested with shrimp of 1.2 to 1.4 g mean weight. There were no significant differences in assimilation of proteins between diets 1 (75.9%), 3 (75.2%) and K (80.9%). Protein assimilation from diets containing no brewer's yeast was statistically greater than values obtained with feeds 18 (58.3%), 25 (53.9%) and 26 (51.3%) containing different proportions of yeast. Unfortunately, complete statistical tests concerning carbohydrate assimilation for these diets were not performed due to the heterogeneity of some of the variances. Consequently, paired comparisons using Student's t tests show that carbohydrates from diet 1 (47.2%) were significantly better assimilated than those from diet 3 (35.9%); carbohydrates from diet K (59.2%) were better assimilated than those from diet 26 (38.0%).

Table 7. Relative Assimilation of Crude Protein and Carbohydrate from Diets 1, 3, K, 18, 11-B, 25 and 26 Fed to *P. stylirostris*. Values are mean ± SEM for number of observations indicated.

Diet	Mean weight (g)	Number of observations[a]	Protein assimilation (%)	Number of observations[a]	Carbohydrate assimilation (%)
1	1.3 ± 0.07	4	75.9 ± 2.91	4	47.2 ± 1.43
3	1.4 ± 0.07	4	75.2 ± 3.61	3	35.9 ± 2.12
K	1.3 ± 0.06	4	80.9 ± 1.97	4	59.2 ± 2.66
18	1.4 ± 0.06	4	58.3 ± 2.34	3	43.2 ± 2.25
11-B	1.3 ± 0.05	4	67.1 ± 2.42	3	49.2 ± 11.42
25	1.4 ± 0.06	3	53.9 ± 2.41	3	53.1 ± 2.35
26	1.2 ± 0.06	4	51.3 ± 2.94	3	38.0 ± 6.74
			F_s = 18.29*		Heteroscedastic

[a]There were 25 shrimp per observation.

* = significant ($P < 0.05$).

Analyses of covariance were performed to determine if there was any relation between protein and carbohydrate assimilation and the size of the shrimp. These analyses revealed that assimilations of both protein (Fig. 1) and carbohydrate (Fig. 2) are related to the size of the shrimp; with a pooled slope for both protein and carbohydrate significant (t = 2.493, 16 df and t = 3.265, 12 df respectively).

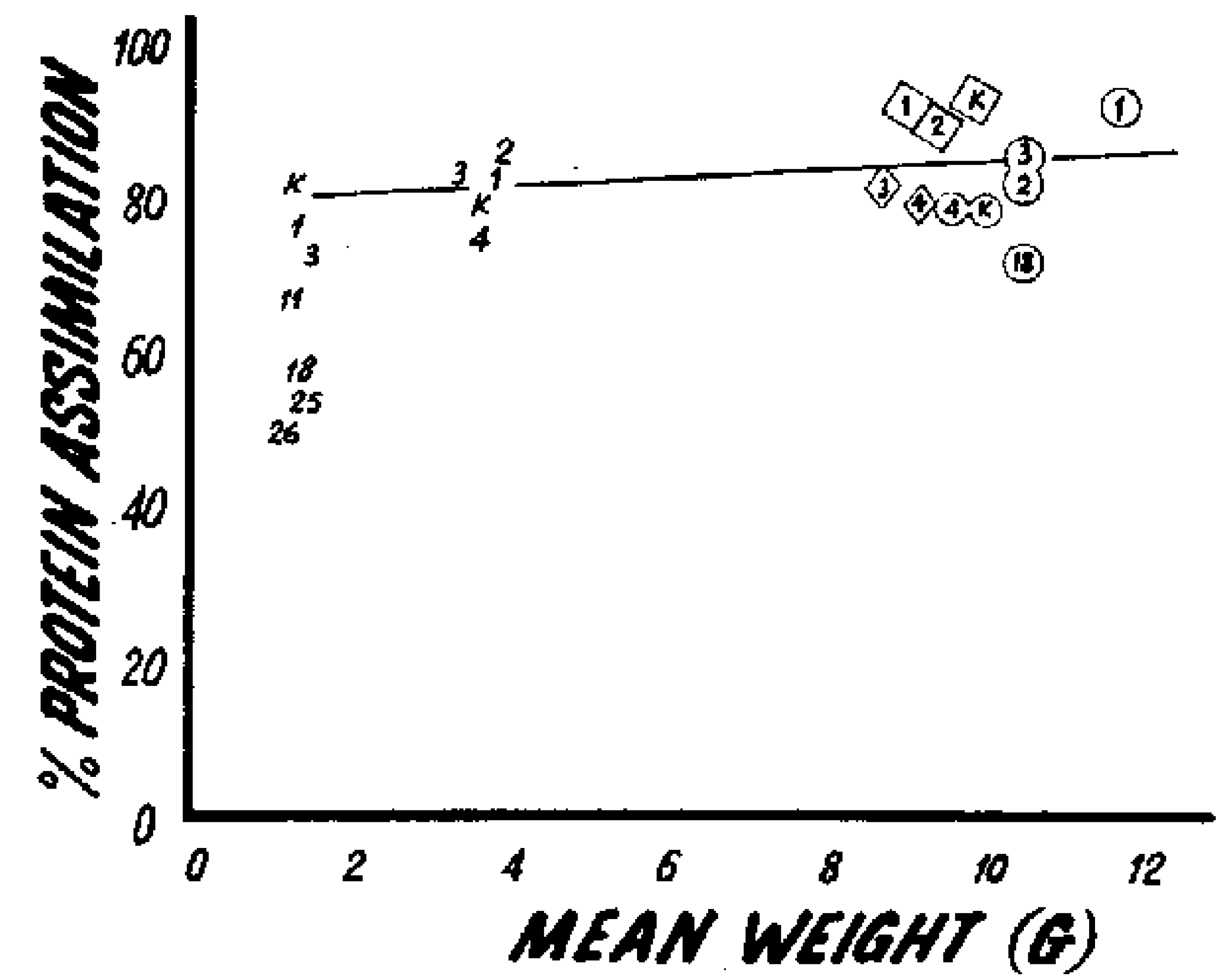

Figure 1. Relationship between the percentage of protein assimilation and the weight of *P. stylirostris*. Symbols represent different experiments. Numbers and letters show diets tested. Line represents pooled regression line for diets 1 to 4 and K.

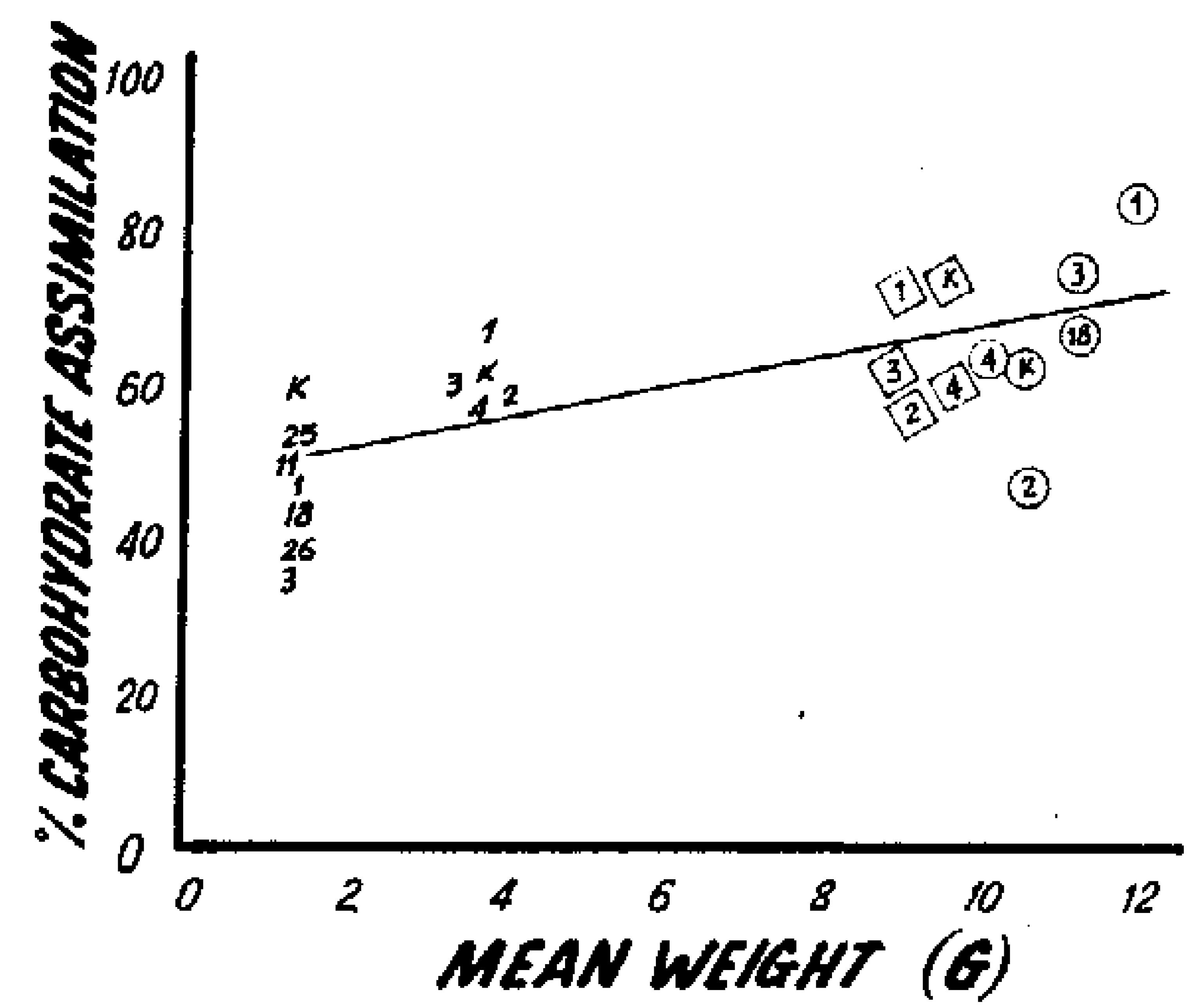

Figure 2. Relation between the percentage of carbohydrate assimilation and the weights of *P. stylirostris*. Symbols represent different trials. Numbers and letters show diets tested. Line represents pooled regression line for diets 1 to 4 and K.

DISCUSSION

Two preliminary experiments were done to determine if there were significant errors in the analysis caused by: 1) leaching of nutrients from the feed, 2) loss of nutrients from the feces due to leaching and/or bacterial degradation, and/or 3) leaching of chromic oxide from the feed and/or feces. The first two would give erroneously high assimilation efficiency values whereas the last one would result in erroneously low values. Results of the two preliminary experiments indicated that these potential sources of error for the determination of assimilation efficiencies are negligible.

Another potential source of error is that the assimilation efficiency value does not take into consideration the influence of metabolic fecal nitrogen (MFN) due to residues of the digestive juices, epithelial cells from digestive tract, bacterial residues and constituents of the peritrophic membrane formed around the fecal pellets (Maynard and Loosli 1969; Forster 1953; Forster and Gabbott 1971). Since the influence of MFN has been proved to have little effect on the fecal protein analysis (Forster and Gabbott 1971; Colvin 1976), this potential source of error was assumed to be insignificant.

The results of the present experiments with the larger *P. stylirostris* (an average weight of about 10 g) indicate that more protein was assimilated from diet 1 containing 12.7% of squid meal than from feeds in which α-soy partially or totally replaced this ingredient. In addition, when α-soy protein from diet 3 was replaced by brewer's yeast (diet 18), protein assimilation was decreased. These data suggest that animal proteins are better assimilated than those of vegetable origin. This agrees with the report that protein from animal sources (frozen small shrimp and fish meal) was better assimilated by *P. japonicus* than protein from a vegetable origin (Nose 1964). However, Ting (1970) concluded that absorption of protein from animal and vegetable sources was similar for *P. monodon*. These results suggest that protein assimilation varies for different species and probably reflects different natural nutritional patterns (e.g., carnivorous vs herbivorous).

Smaller shrimp (approximately 1 to 4 g mean weight) showed no significant difference between the relative assimilation of proteins from squid meal and α-soy. Since this assimilation pattern is different from that found in larger animals, there may be a change in digestive and absorbtive ability with the size of the shrimp. Also, it was observed that the apparent digestion and absorption of proteins was significantly lower in those diets in which all of the α-soy or part of the shrimp meal was replaced by brewer's yeast.

In all the experiments, carbohydrates were less efficiently assimilated than were proteins. These results agree with those obtained by Condrey et al. (1972) who used the ash method to determine assimilation for *P. setiferus* and *P. aztecus*. However, for the caridean species *Palaemon serratus* and *Pandalus platyceros*, assimilation efficiencies for carbohydrates and proteins from formulated diets were similar (Forster and Gabbott 1971; Forster 1972). Differences in assimilation of carbohydrates and proteins by *P. stylirostris* could be due to a relatively greater activity of proteases or to low cellulase activity.

Carbohydrates from diet 1 were better assimilated than those provided by diet 3 containing 12.7% α-soy and no squid meal. A possible

explanation for the diet containing the highest amount of α-soy showing the lower carbohydrate assimilation could be an inhibition of the shrimp carbohydrases by some factor in the α-soy. Soy meal has been reported to contain a trypsin inhibitor which also decreases the amylase activity (Khayambashi and Lyman 1966). The positive linear relation determined between shrimp size and protein and carbohydrate assimilation may indicate a more developed enzymatic system in larger shrimp.

After a 7-day adjustment period a slight, though not significant, increase in protein assimilation was obtained with no differences in protein assimilation for all diets. Since there were differences in protein assimilation before the adjustment period these results suggest enzymatic adaptation to the diets with time. An enzymatic adaptation is also indicated by a significant increase in carbohydrate assimilation after the 7-day adaptation period.

Fenucci et al. (1980), using similar size animals (approximately 4 g) of *P. stylirostris* and the same diets (1, 2, 3, 4 and K) used in this study (Table 6), showed that diets 2 and 4 gave the best food conversion ratios and growth. In this study, diet 2 definitely did not give the best protein and carbohydrate assimilation efficiencies and diet 4 gave the lowest protein and carbohydrate assimilation values of the 5 diets (Table 6). This lack of correlation between assimilation and growth data could mean that the limiting factor for growth in these diets was not the factor for which assimilation efficiencies were determined. Colvin (1976) also found no correlation between protein assimilation efficiencies and the growth of *P. indicus*.

In conclusion, differences in protein assimilation according to the sources and changes in assimilation efficiencies with the size of the animals and time of exposure to the diets have been observed. Further, the lack of relationship between protein or carbohydrate assimilation and the growth of the shrimp indicates that the response of the animals to the diets can be due to other factors such as fatty acids, vitamins, minerals, etc., instead of protein and carbohydrate levels in the diets.

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